Docket No.: 1516-0126PUS1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Masaru YAMAKOSHI et al.

Application No.: 10/509,120 Confirmation No.: 3292

Filed: November 29, 2004 Art Unit: 1655

For: METHOD OF DETECTING MILD IMPAIRED GLUCOSE TOLERANCE OR INSULIN SECRETORY DEFECT Examiner: P. C. Martin

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, Masaru YAMAKOSHI and Takuji KOUZUMA, declare the following:

1. We are citizens of Japan, residing at 20-6, Nishikigaoka, Mishima-shi, Shizuoka 411-0808, Japan and 4671-2, Wakamatsu-cho, Mishima-shi, Shizuoka 411-0025, Japan, respectively.

2. We are named co-inventors of the above-identified application.

3. We have read and understand the specification and claims to the above-identified application along with the outstanding Office Action and the cited references: Ashizawa et al., *Journal of Biophysical Methods*, Vol. 44, pp. 89-94, 2000 (hereinafter, "Ashizawa et al.") and Tazoe et al., U.S. Patent No. 6,309,852 (hereinafter, "Tazoe et al.") and Kozuma et al., U.S. Patent No. 6,046,018 (hereinafter, "Kozuma et al.").

Specifically, Ashizawa et al. disclose the use of ATP-hexokinase to eliminate glucose in an assay for determining the amount of myo-inositol. Tazoe et al. disclose the use of ADP-hexokinase, phosphohexose isomerase (PHI) and 6-phosphofructokinase (6-PFK) for the purpose of eliminating glucose from the assay by conversion of glucose to fructose-1,6-diphosphate *via* three enzymatic steps. If one of ordinary skill in the art were to combine the teachings of these two references, one would derive a four-enzyme system including ATP-hexokinase, ADP-hexokinase, PHI and 6-PFK. This hypothetical composition taught by the two cited references is different from the presently claimed invention.

The hexokinase reaction is as follows:

glucose + ATP → glucose-6-phosphate (hereinafter, "G-6-P") + ADP

glucose + ADP → glucose-6-phosphate + AMP

The disclosure of Tazoe et al. shows conversion of glucose to G-6-P and G-6-P to fructose-6-phosphate and then fructose-6-phosphate to fructose-1,6-diphosphate, as shown below:

Glucose

↓ ATP-hexokinase, ADP-hexokinase, or ATP-glucokinase

G-6-P + ADP, or AMP

↓ PHI

fructose-6-phosphate

↓ 6-PFK

fructose-1,6-diphosphate

Tazoe et al. disclose or suggest removal of glucose using hexokinase which results in the unfavorable formation of large quantities of G-6-P and ADP. Because Tazoe refers to ADP in an

unfavorable product, Tazoe et al. must use ATP-hexokinase to perform this reaction, not ADP-hexokinase. Since this reaction is reversible, accumulation of G-6-P and ADP interferes with the removal of glucose as the product recombines to reform glucose and ATP. Thus, Tazoe et al. attempts to remove G-6-P from the reaction system. In contrast, the presently claimed invention is directed at removing ADP from the system to prevent the accumulation of ADP, not G-6-P.

Tazoe et al. describe preventing reversal of the conversion of the glucose to G-6-P by further conversion of the G-6-P. Tazoe et al. do not disclose or suggest the elimination of ADP from the reaction system or that doing so would be useful in eliminating glucose.

We actually attempted to perform the reaction of Tazoe et al. but the reactions were unsuccessful.

ATP-hexokinase and ADP-hexokinase were removed from the compositions of the presently claimed invention and substituted with PHI and 6-PFK. Further, G-6-P was used instead of glucose to confirm if sufficient elimination of G-6-P was possible using the methods and reagents according to Tazoe et al.

Samples were prepared as follows: a first set of samples containing 200 μM myo-inositol alone were prepared, and a second set of samples containing 200 μM myo-inositol in addition to 556 mM G-6-P (corresponding to 10 g/dL glucose) were prepared.

	6-PFK concentration (U/mL)	
	0	10
200 μM myo-inositol	30.8*	31.4*
200 μM myo-iunositol + 556mM G-6-P	23.5*	16.9*
Relative ratio (%)	76.1	53.7

*Unit: mABS/min

The interference by G-6-P was calculated as "100 – 53.7 = 46.3%" at 10 U/mL of 6-PFKJ. The method and reagents according to Tazoe et al. appeared to be utterly unable to allow

determination of myo-inositol according to the above data. It is uncertain why, when using the methods and reagents of Tazoe et al., the relative ratio was 76.1% at 0 U/mL of 6-PFK.

In stark contrast to this failed composition, the presently claimed compositions enabled elimination of sufficient glucose to yield a very low interference of only 11.0% according to the data presented below. In the data presented below, samples comprising 200 μM myo-inositol/10g/dL glucose were assayed.

200 μM myo-inositol	32.2*
200 μM myo-inositol / 10 g/dL glucose	28.7*
Relative ratio (%)	89.0

*Unit: mABS/min

As shown above, combinations of the references yield completely inoperable embodiments. Thus, the references, when considered in combination, actually teach away from the presently claimed compositions.

The presently claimed compositions are quite effective at eliminating glucose up to the concentration of 10 g/dL as shown in Example 3, and Figure 3, of the present specification. On the other hand, the compositions of Tazoe et al. were only minimally effective up to a concentration of approximately 2 g/dL as shown in Examples 4 and 6 of Tazoe et al. The result of the invention is not expected by one of ordinary skill in the art who read Tazoe et al. with Ashizawa et al.

Claim 21 and 24 are also rejected by the Examiner over the combination of Ashizawa et al., Tazoe et al. and Kozuma et al. For the reasons explained above in relation to the combination of Ashizawa et al. with Tazoe et al., a composition comprising:

thio-NAD;

NADH;

myo-inositol dehydrogenase; and

ATP-hexokinase and/or ADP-hexokinase

provides a result that would not be expected by one of ordinary skill in the art who reads Ashizawa et al., Tazoe et al. and Kozuma et al.

I hereby declare that all statements made herein of any own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 9/19 2006 Masaru Yamakoshi

Masaru YAMAKOSHI

Dated: 9/19 2006 Takuji Kouzuma

Takuji KOUZUMA